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In vitro and *in vivo* antiproliferative activity of *Calotropis procera* stem extra

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ABSTRACT

The cytotoxic potential of stem organic extracts from *Calotropis procera* (Asclepiadaceae) was firstly evaluated agai cancer cell lines by MTT assay. Subsequently, samples considered cytotoxic were tested for antimitotic activity sea urchin egg development and for *in vivo* antiproliferative activity in mice bearing Sarcoma 180 tumor. Amo the five extracts (hexane, dichloromethane, ethyl acetate, acetone and methanol), ethyl acetate and acetone extra displayed higher cytotoxic potential against tumor cells, with IC_{50} ranging from 0.8 to 4.4 μg/mL, while methano extract was weakly cytotoxic. Cytotoxic extracts also exhibited cell division inhibition capacity by antimitotic ass revealing IC_{50} values lower than 5 μg/mL. In the *in vivo* antitumor assessments, ethyl acetate- and acetone-treat animals showed tumor growth inhibition ratios of 64.3 and 53.1%, respectively, with reversible toxic effects on li and kidneys. Further studies are in progress in order to identify *C. procera* cytotoxic compound(s) and to understa the mechanism of action responsible for this tumor-decreasing potential.

Key words: antimitotic, antiproliferative, *Calotropis procera*, Sarcoma 180 tumor, stem extracts.

INTRODUCTION

Calotropis procera Aiton, 1811 (Gentianales: Asclepiadaceae) is a perene Asian shrub called "Ushar", being very common in adverse climate conditions and poor soils explaining its good adaptation to the Northeast Brazil, where it was introduced at the beginning of the century XIX, spreading within different biomes such as "Caatinga" and "Cerrado" (Kissmann and Groth 1999, Lorenzi and Matos 2002).

de seda", "leiteiro", "queimadeira" and "ciúme" mann and Groth 1999, Lorenzi and Matos 2002). ent parts of this tree have been used to treat and diseases. In India, the leaves are prepared to ea from a variety of conditions (Satyavati et al. 1976) possess anthelmintic (Iqbal et al. 2005), analge tipyretic, antispasmodic (Mascolo et al. 1988, et al. 2000) and anti-inflammatory (Kumar an 1994) properties. It is also notorious their anti

bly reached due to flavonoids with antioxidant potentiality similar to vitamin C (Ahmed et al. 2003, Setty et al. 2007, Ferreira et al. 2008).

The latex found in the aerial parts (young leaves, predominantly) is utilized to treat diarrhea (Satyavati et al. 1976), possibly due to its desensitization of the gastrointestinal smooth muscle cells (Kumar and Shivkar 2004) and antimicrobial action (Jain et al. 1996). Moreover, latex possess biological activity against the coccidian protozoa *Eimeria ovinoidalis* (Mahmoud et al. 2001) and *Aedes aegypti* larvae (Ramos et al. 2006), protection against hyperglycemia induced by alloxan (Roy et al. 2005) and anti-inflammatory (Alencar et al. 2004) effects. Root methanolic extracts simulates oestrogenic actions, altering uterine endometrial and interfering on the blastocyst implantation (Kamath and Rana 2002), while stem bark extracts reduces bronchial inflammation, showing antitussigene activity by oral administration (Dieye et al. 1993). In this work, the great pharmacological potential of *C. procera* was evaluated aiming to study the *in vitro* cytotoxicity of stem extracts and their *in vivo* antitumor capacity on mice transplanted with Sarcoma 180 cells.

MATERIALS AND METHODS

Plant Material and Extract Preparation

Calotropis procera samples were collected in Sobral, Ceará, Brazil. A voucher specimen (34.706) was authenticated by Dr. Edson de Paula Nunes and deposited at Prisco Bezerra Herbarium (EAC), Department of Biology of Universidade Federal do Ceará, Brazil.

Stem samples (1000.0 g) were firstly grounded to a fine powder, weighed and subjected to successive extractions (4×) in 100% ethanol with a sample mass to solvent volume proportion of 1:5 at room temperature (25°C). Then, the material was concentrated under pressure until turn out a viscous residue. Afterwards, the ethanolic extract was partitioned using growing polarity organic solvents: hexane, dichloromethane, ethyl acetate, acetone and methanol. Each extraction procedure was performed under shaking for 24 h. The ex-

Animals

Adult Swiss mice (*Mus musculus* Linnaeus, 1758) were obtained from the animal facilities of Universidade Federal do Ceará. They were kept in cages (ALESCO, São Paulo) under standard conditions of light (12 h with alternative day and night cycles) and temperature (25 ± 1°C), and were housed with access to commercial rodent stock diet (Purina, São Paulo, Brazil) and water *ad libitum*. The investigational protocol was approved by the local Ethical Committee in Animal Research at Universidade Federal do Ceará (Process No. 102/2007), and is in accordance with International Standard on the care and use of experimental animals (EEC Directive of 1986, 86/609/EEC).

Adult sea urchins of the *Lytechinus variegates* Lamarck, 1816 (Echinoidea: Toxopneustidae) species were collected at Pecém beach, northeastern coast of Ceará, Brazil. The urchins were maintained under standard laboratory conditions until the beginning of the experiments.

MTT Assay

The cytotoxicity against the tumor cell lines HL-60, CEM (human leukemia), HCT-8 (human colon cancer) and B-16/F10 (murine melanoma) was determined by MTT assay (Mosmann 1983), which analyzes the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a purple formazan product. All lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penincillin and 100 μg/mL streptomycin, at 37°C with 5% CO_2. Briefly, cells were plated in 96 well plates (0.7×10^5 cells/well for adherent cells and 0.3×10^5 cells/well for suspended cells) and incubated to allow cell adhesion. Twenty-four hours later, stem extracts (hexane, dichloromethane, ethyl acetate, acetone and methanol) were added to each well (0.39–25 μg/mL). After 72 h of incubation, the supernatant was replaced by fresh medium containing 10% MTT. The formazan product was dissolved in DMSO to measure absorbance

ANTIMITOTIC ASSAY

The antimitotic assay was performed in 24-well plates according with Moreira et al. (2007). Gamete elimination from *L. variegatus* was induced by injecting 3.0 mL of 0.5 M KCl into the urchin's coelomic cavity via the periostomial membrane. Concentrated sperm was collected with a Pasteur pipette and preserved under low temperature until the fertilization. Each well received 1 mL of fertilized egg suspension and extracts (ethyl acetate, acetone and methanol) were added immediately after fertilization (within 2 min) (0.1, 1, 5 and 10 μg/mL). Doxorubicin (0.3 μg/mL) and 1.6% DMSO were used as positive and negative controls, respectively. Plates were then shaken in a constant temperature water bath at $26 \pm 2°C$. At appropriate times, aliquots of 100 μL were fixed with 10% formaldehyde to obtain first and third cleavages and blastulae. One hundred eggs or embryos per well were counted in order to calculate IC_{50} values.

In vivo ANTITUMOR EVALUATION

Fifty healthy male mice (*M. musculus*) weighing 23–26 g were subcutaneously implanted with nine-day-old Sarcoma 180 ascites tumor cells (2×10^6 cells/0.5 mL) into the left hind groin of the mice. On the next day, they were randomly separated into five groups (n = 10 each) to receive stem extracts (ethyl acetate, acetone and methanol dissolved in 0.9% saline) at the dose of 250 mg/kg/day. In contrast, negative and positive controls received saline and 5-FU (50 mg/kg/day), respectively, all administered intraperitoneally for 7 days.

On day 8, mice were sacrificed by cervical dislocation and their organs (kidneys, spleens and livers) and tumors were dissected out, grossly examined for size, color changes and hemorrhage, weighed and preserved in 10% formaldehyde solution. The inhibition ratio of tumor growth (%) was calculated by the following formula: inhibition ratio (%) $= [(A - B)/A] \times 100$, where A is the average tumor weight of the negative control, and B is the tumor weight of the treated group. To examine morphological changes by light microscopy (Olympus, Tokyo, Japan), small pieces of organs and tumors

STATISTICAL ANALYSIS

For cytotoxicity assays, the IC_{50} values and the confidence intervals were obtained by nonlinear sion using the Graphpad program (Intuitive Softw Science, San Diego, CA). In order to determine ences among the treatments, data (mean ± standa mean) were compared by one-way analysis of v (ANOVA) followed by Newman-Keuls test ($P <$

RESULTS AND DISCUSSION

Researches for antineoplasic compounds have d strated the great pharmacological relevance of th extracts (Cragg and Newman 2005, Costa et al. Buriol et al. 2009). At the last decades, *C. p* received special attention, with lots of publicati scribing the biological activities of molecules an ous and organic extracts obtained from its disti sues (Jain et al. 1996, Kamath and Rana 2002, et al. 2003, Iqbal et al. 2005, Ramos et al. 200 et al. 2007).

In the present work, we firstly determined th toxic activity of organic extracts from *C. proce* using the MTT assay. According to the Americ tional Cancer Institute, the IC_{50} limit to consider a ising crude extract for further purification is low 30 μg/mL (Suffness and Pezzuto 1990). Among extracts, ethyl acetate and acetone showed hig totoxic potential against tumor cells, with IC_{50} r from 0.8 to 4.4 μg/mL for colon (HCT-8) and me (B-16) cells, respectively (Table I). Methanolic was weakly cytotoxic, despite the fact that it d strate moderately good activity on CEM line [IC_5 of 2.8 (2.1–4.1)μg/mL].

Previously, some reports showed that d parts of the plant exhibit cytotoxicity on cance (Smit et al. 1995, Van Quaquebeke et al. 2005, C et al. 2007). Van Quaquebeke et al. (2005) isc natural cardiotonic steroid from the methanolic of *C. procera* root barks called 2″-oxovoruscha developed a new hemisynthetic cardenolide de named UNBS1450 which display *in vitro* antipr

TABLE I
Cytotoxic activity of extracts obtained from *Calotropis procera* stem on tumor cell lines after 72 h of exposure.

Substance	Yield (%)	Cell line			
		IC_{50} (μg/mL)*			
		HL-60	CEM	B-16/F10	HCT-8
Hexane	5.3	> 25	> 25	> 25	> 25
Dichloromethane	22.7	> 25	> 25	> 25	> 25
Ethyl acetate	11.6	1.6	1.4	2.0	2.5
		1.4–1.9	1.1–3.8	1.0–3.9	2.3–2.6
Acetone	10.4	2.1	1.4	4.4	0.8
		2.1–2.2	1.3–2.8	2.1–9.0	0.6–1.0
Methanol	50.0	8.2	2.8	> 25	10.2
		5.4–12.4	2.1–4.1		7.2–14.2
Doxorubicin	—	0.02	0.02	0.002	0.01
		0.01–0.02	0.02–0.03	0.001–0.003	0.01–0.02

*Data are presented as IC_{50} values and 95% confidence intervals for human leukemia (HL-60, CEM), murine melanoma (B-16/F10), and human colon cancer (HCT-8) cells. Experiments were performed in triplicate.

fragmentation and cell volume reduction (Choedon et al. 2006, Oliveira et al. 2007). It is established that cardiotonic steroid glycosides (bufalin and digoxin, for instance) are capable to kill cancer cells through the activation of apoptotic pathways (McConkey et al. 2000, Kurosawa et al. 2001). On the other hand, additional and recent investigations propose autophagy as a probable kind of cell death caused by UNBS1450 in human glioblastoma lines (Lefranc et al. 2008). Autophagy is a singular self-destructive process in which injured, unnecessary or old parts of the cells, as mitochondria and endoplasmic reticulum, are degraded by enzymatic activity within lysossomes (Maiuri et al. 2007).

Sea urchin egg development shows some peculiarities that allows us to suggest how antimitotic substances act. Acetone, ethyl acetate and methanol extracts inhibited the division of sea urchin eggs since the first cleavage in a concentration-dependent way, revealing IC_{50} values lower than 5 μg/mL (Table II). The inhibition at the first cleavage is related to DNA and/or protein synthesis or microtubule assembly, given that RNA synthesis is very slow or absent after fertilization. At this time, the rapid increasing in the rate of protein synthesis is corresponding to nucleus duplication can be observed in the cytoplasm. Since zygotes treated with the extracts exhibited homogeneous cytoplasm, this process appears not to have been affected (Jacobs and Wilson 1986). So, the stem organic extracts acetone, ethyl acetate and methanol may affect DNA and/or protein synthesis, confirming their *in vitro* antiproliferative activity showed by MTT assay.

TABLE II
Inhibition of cell division of *Calotropis procera* stem extracts on embryos of the sea urchin *Lytechinus variegatus* on the first and third cleavage and blastulae stages.

Substance	IC_{50} (μg/mL)*		
	First cleavage	Third cleavage	Blastulae
Ethyl acetate	3.2	2.0	1.1
	2.7–3.7	1.5–2.7	1.1–1.2
Acetone	3.7	2.7	3.5
	2.5–5.5	1.2–3.6	1.5–8.0
Methanol	4.1	3.7	4.7
	3.7–4.4	3.3–4.2	4.1–5.5
Doxorubicin	6.3	0.3	0.5
	4.3–9.1	0.2–0.7	0.3–1.0

TABLE III
Effects of the *Calotropis procera* stem extracts on mice transplanted with Sarcoma 180 cells.

Group	Dose (mg/kg/day)	Animal weight (g)	Liver	Kidneys	Spleen	Tumor (g)	Tumor inhibition (%
			g/100g body weight				
Control	—	30.00 ± 0.50	5.94 ± 0.21	1.43 ± 0.07	0.69 ± 0.05	3.25 ± 0.47	—
5-FU	50	24.60 ± 0.40[a]	4.86 ± 0.27	1.28 ± 0.03	0.22 ± 0.01[a]	0.11 ± 0.05[a]	96.5
Ethyl acetate	250	29.00 ± 0.94	4.93 ± 0.09	1.38 ± 0.02	0.46 ± 0.03	1.40 ± 0.35[a]	64.3
Acetone	250	29.38 ± 0.82	5.20 ± 0.16	1.50 ± 0.06	0.49 ± 0.03	1.34 ± 0.22[a]	53.1
Methanol	250	31.43 ± 0.92	5.94 ± 0.26	1.39 ± 0.05	0.52 ± 0.08	3.26 ± 0.52	3.22

*Data are means ± S.E.M., n = 10 animals/group, treated for seven days by intraperitoneal route. Positive and negative controls treated by 5-Flouoruracil (5-FU) and saline 0.9%, respectively. [a]P < 0.01 compared to control by ANOVA followed by Newman-Keul

Herein, we also reported the antitumor activity of organic extracts (ethyl acetate, acetone and methanol) obtained from *C. procera* stem in mice bearing Sarcoma 180 tumor. Sarcoma 180 is a mouse-derived tumor very exploited in antitumor research *in vivo* (Sato et al. 2005, Magalhães et al. 2006). The effects of the ethyl acetate, acetone and methanol extracts on tumor growth are described in Table III. A significant reduction in tumor weight ($P < 0.01$) was found at 250 mg/kg/day in both ethyl acetate- and acetone-treated animals (1.40 ± 0.35 g and 1.34 ± 0.22 g, respectively) in comparison with negative control (3.25 ± 0.47 g), leading to tumor growth inhibition ratios of 64.3 and 53.1%, respectively. The dose of 50 mg/kg/day reduced tumor weight in 96.5% in 5-FU-treated mice. In contrast, methanol extract was unable to avoid tumor augmentation within identical experimental conditions.

The mice intraperitoneal treatment with the extracts was not able to interfere on the final body weight and in relative liver, kidneys and spleen weights of the experimental groups (Table III) when compared to negative control ($P > 0.05$). Moreover, neither mortality nor morbidity were recorded during the whole experiment.

Tumor morphological examination of the control group showed large and polygonal cells, with pleomorphic shapes, hyperchromatic nuclei, binucleation, mitosis and muscle invasion (Fig. 1A). Meanwhile, tumors excised from mice treated with 5-FU, ethyl acetate and acetone extracts exhibited extensive areas of coagulative necrosis alternated with pleormorphic cells (Figs.

fer cell hyperplasia, ballooning degeneration o
tocytes, portal tract and centrolobular venous c
tion and discrete microvesicular steatosis, though
eas of necrosis were observed (Figs. 2C, 2D a
Focal infiltrate of inflammatory cells was more
in the methanol group. Regarding kidneys, it w
served glomerular and tubular hemorrhage and
pic changes of the proximal tubular ephitelium i
acetate- and acetone-treated groups (Figs. 3C a
respectively), but the glomerular structure was
tially preserved. On the other hand, methanolic
did not present kidney alterations while 5-FU gro
sented hyaline cylinders (Fig. 3B). There was a
erable hyperplasia of the splenic white pulp and
karyocytes in all treated groups in comparison
negative control (data not shown), a result p
caused by substances found in the extracts.
ethanolic extracts of flowers, buds and roots of
cera contain alkaloids, phenolic compounds/t
saponins and lectins, which may have immunon
tory properties (Imboden 1988, Mossa et al. 199
et al. 2001, Ferreira et al. 2007, 2009).

The kidney and liver have been proposed
major key organs to metabolize environmental tox
stances. Due to the good liver regeneration ca
even when necrosis is found with conjunctive tiss
servation, generally there was a complete hepa
toration (Kumar et al. 2004). Portal tract and cen
ular venous congestion were also visualized in th
trol group, suggesting that these effects are rel

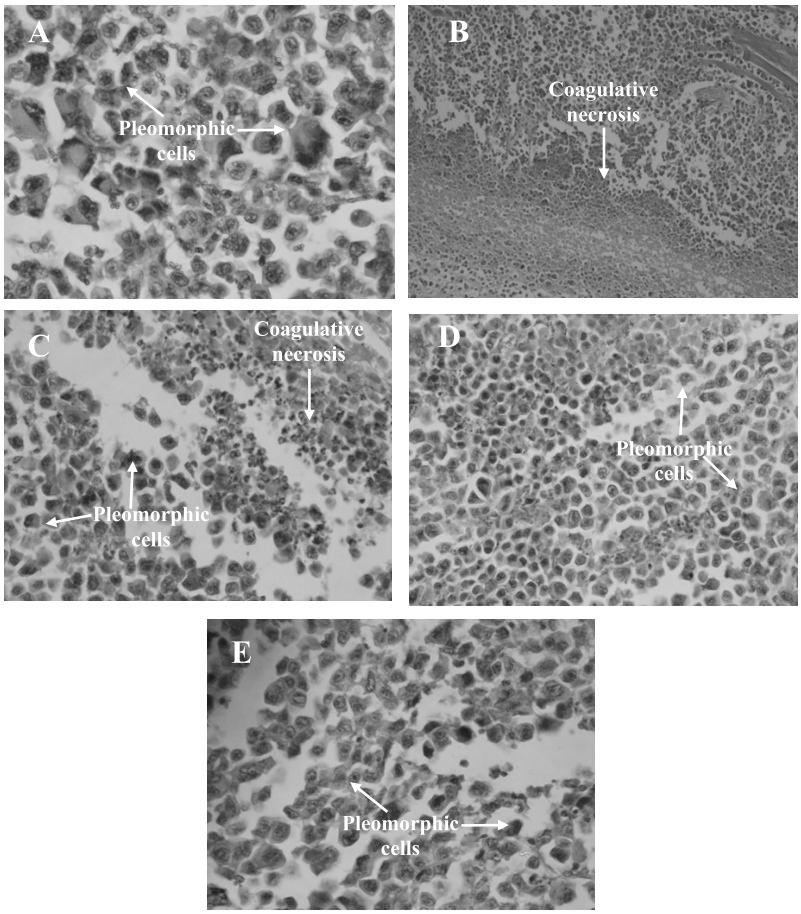

Fig. 1 – Histopathology of tumors excised from Sarcoma 180 transplanted mice after 7 days of intraperitoneal treatment with *Calotropis procera* stem extracts at the dose of 250 mg/kg/day (C – ethyl acetate; D – acetone; E – methanol). Negative control (A) and positive control (B) received 0.9% saline and 5-FU (50 mg/kg/day), respectively. Magnification, 400×.

(Kumar et al. 2004), as seen in 5-FU-treated mice. Although all alterations observed in liver and kidney-treated animals were considered reversible, the kidneys should be the target organ of the ethyl acetate and acetone extracts. Nevertheless, the reversible character of injuries proposes that treatment removal leads to quick improvement (Scheuer and Lefkowitch 2000). Melo et al. (2001) showed that goats subcronically fed with pulverized dehydrated aerial parts of *C. procera* (leaves and twigs) did not undergo suggestive biochemical modifications of liver damage, which could explain the light to 3 g/kg) do not produce any visible toxic symptoms or mortality, while extended treatment (90 days) causes significantly higher toxicity (Mossa et al. 1991).

The present work shows that ethyl acetate, acetone and methanol stem extracts from *C. procera* possess promising *in vitro* antiproliferative activity on cancer lines and sea urchin eggs. Meanwhile, ethyl acetate and acetone extracts are able to reduce *in vivo* tumor growth of Sarcoma 180 transplanted mice in the presence of liver and kidneys reversible toxic effects. Some investigations are in progress in order to identify *C. pro-*

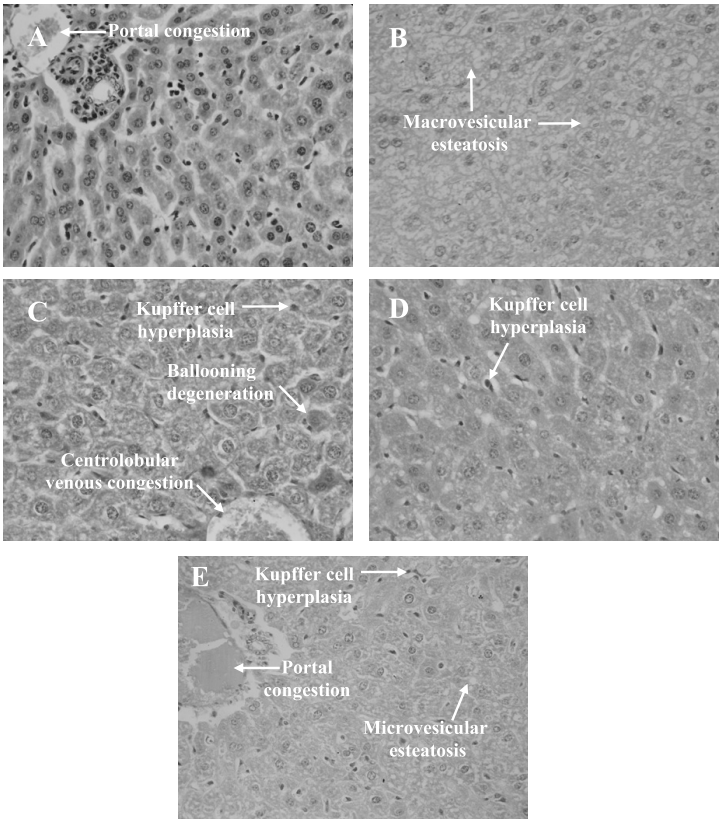

Fig. 2 – Histopathology of livers excised from Sarcoma 180 transplanted mice after 7 days of intraperitoneal treatment with *Calotropis* stem extracts at the dose of 250 mg/kg/day (C – ethyl acetate; D – acetone; E – methanol). Negative control (A) and positive control (B) 0.9% saline and 5-FU (50 mg/kg/day), respectively. Magnification, 400×.

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RESUMO

O potencial citotóxico de extratos orgânicos do caule de *Calo-*

sequentemente testadas para atividade antimitótica
desenvolvimento de ovos de ouriço-do-mar e para a
antiproliferativa *in vivo* em camundongos transplantad
tumor Sarcoma 180. Dentre os cinco extratos estudad
xano, diclorometano, acetato de etila, acetona e meta
extratos acetato de etila e acetona mostraram maior p
citotóxico contra células tumorais, com CI_{50} variand
to 4,4 μg/mL, enquanto o extrato metanólico revelou
camente citotóxico. Os extratos citotóxicos também e
capacidade de inibição da divisão celular com valores
menores que 5 μg/mL. Nas avaliações antitumorais
os animais tratados com os extratos acetato de etila e
mostraram taxas de inibição do crescimento tumoral d
53,1%, respectivamente, com efeitos tóxicos reversíve

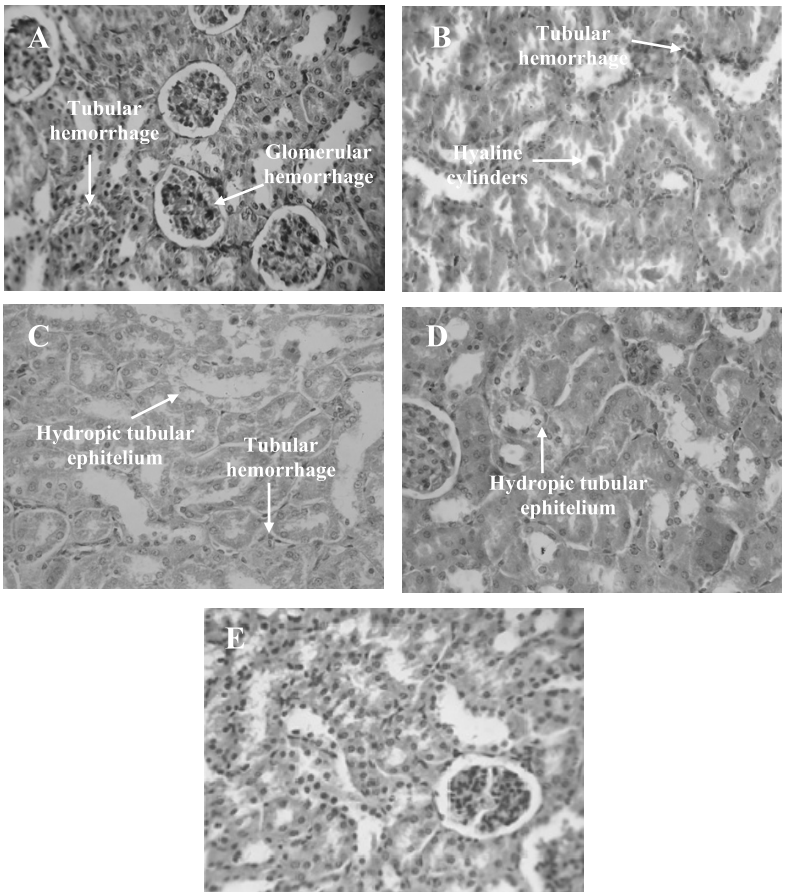

Fig. 3 – Histopathology of kidneys excised from Sarcoma 180 transplanted mice after 7 days of intraperitoneal treatment with *Calotropis procera* stem extracts at the dose of 250 mg/kg/day (C – ethyl acetate; D – acetone; E – methanol). Negative control (A) and positive control (B) received 0.9% saline and 5-FU (50 mg/kg/day), respectively. Magnification, 400×.

REFERENCES

AHMED MKK, RANA AC AND DIXIT VK. 2003. Free radical scavenging activity of Calotropis species. Indian Drugs 40: 654–655.

ALENCAR NMN, OLIVEIRA JS, RIBEIRO RA AND RAMOS MV. 2004. Anti-inflammatory effect of the latex from *Calotropis procera* in three different experimental models: Peritonitis, Paw edema and Hemorrhagic cystitis. Planta Med 70: 1144–1149.

BRANDHORST PB. 1985. Informational content of the echinoderm egg. In: BROWDER LW (Ed), Development Biology, a Comprehensive Synthesis Oogenesis, New York: Plenum Press, p. 525–576.

CHOEDON T, MATHAN G, ARYA S, KUMAR VL AND KUMAR V. 2006. Anticancer and cytotoxic properties of the latex of *Calotropis procera* in a transgenic mouse model of hepatocellular carcinoma. World J Gastroenterol 12: 2517–2522.

COSTA PM, FERREIRA PMP, BOLZANI VS, FURLAN M, SANTOS VAFFM, CORSINO J, MORAES MO, COSTA-LOTUFO LV, MONTENEGRO RC AND PESSOA C. 2008. Antiproliferative activity of pristimerin isolated from *Maytenus ilicifolia* (Celastraceae) in human HL-60 cells. Toxicol *in vitro* 22: 854–863.

CRAGG GM AND NEWMAN DJ. 2005. Plants as a source of anti-cancer agents. J Ethnopharmacol 100: 72–79.

DIEYE AM, TIDJANI MA, DIOUF A, BASSENE E AND FAYE B. 1993. Senegalese pharmacopoeia: study of acute toxicity and antitussive activity of *Calotropis procera* AIT (Asclepiadaceae). Dakar Med 38: 69–72.

EEC DIRECTIVE OF 1986. Council Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (86/609/EEC).

FERREIRA PMP, CARVALHO AFFU, SOUSA DF, MAGALHÃES JF, MARTINS AR, MARTINS MAC AND QUEIROZ MGR. 2007. Water extract of *Moringa oleifera* seeds: a toxicological approach. REPM 1: 45–57.

FERREIRA PMP, FARIAS DF, OLIVEIRA JTA AND CARVALHO AFFU. 2008. *Moringa oleifera*: Bioactive compounds and nutritional potential. Rev Nutr 21: 431–437.

FERREIRA PMP, CARVALHO AFFU, FARIAS DF, CARIOLANO NG, MELO VMM, QUEIROZ MGR, MARTINS AMC AND MACHADO-NETO JG. 2009. Larvicidal activity of the water extract of *Moringa oleifera* seeds against *Aedes aegypti* and its toxicity upon laboratory animals. An Acad Bras Cienc 81: 207–216.

IMBODEN JB. 1988. The regulation of intracellular signals during lymphocyte activation. Immunol Today 9: 17–18.

IQBAL Z, LATEEF M, JABBAR A, MUHAMMAD G AND KHANA MN. 2005. Anthelmintic activity of *Calotropis procera* (Ait.) Ait. F. flowers in sheep. J Ethnopharmacol 102: 256–261.

JACOBS RS AND WILSON L. 1986. Fertilized sea urchin egg as a model for detecting cell division inhibitors. In: ASZALOR A (Ed), Modern analysis of antibiotics, New York, Marcel Dekker, Inc., p. 481–493.

JAIN SC, SHARMA R, JAIN R AND SHARMA RA. 1996. Antimicrobial activity of *Calotropis procera*. Fitoterapia 67: 275–277.

KAMATH JV AND RANA AC. 2002. Preliminary study on antifertility activity of *Calotropis procera* roots in female rats. Fitoterapia 73: 111–115.

KISSMANN KG AND GROTH D. 1999. Plantas infestantes e nocivas, São Paulo, Editora BASF, 523 p.

KUMAR V, ABBAS AK, FAUSTO N, ROBBINS SL AND COTRAN RS. 2004. Pathology Basis of Disease. China: WB Saunders, 1552 p.

KUMAR VL AND SHIVKAR YM. 2004. *In vivo* and effect of latex of *Calotropis procera* on gastroi smooth muscles. J Ethnopharmacol 93: 377–379

KUROSAWA M, TANI Y, NISHIMURA S, NUMAZ AND YOSHIDA T. 2001. Distinct PKC isozym late bufalin-induced differentiation and apoptosi man monocytic cells. Am J Physiol Cell Phys 459–464.

LEFRANC F ET AL. 2008. Targeting the alpha 1 su the sodium pump to combat glioblastoma cells. surgery 62: 211–221.

LORENZI H AND MATOS FJA. 2002. Plantas medic Brasil: nativas e exóticas. São Paulo, Instituto Pla 544 p.

MAGALHÃES HIF, VERAS ML, TORRES MR, APNN, PESSOA ODL, SILVEIRA ER, COSTA-L LV, MORAES MO AND PESSOA C. 2006. *In vitr vivo* antitumor activity of physalins B and D from *angulata*. J Pharm Pharmacol 58: 235–241.

MAHMOUD OM, HAROUN EM, SOBAIH MA, OM AND ADAM SEI. 2001. Comparative efficacy *tropis procera* latex and sulfadimidine against expe ally-induced *Eimeria ovinoidalis* infection in Najd Small Ruminant Res 42: 135–140.

MAIURI MC, ZALCKVAR E, KIMCHI A AND KROE 2007. Self-eating and self-killing: crosstalk betw tophagy and apoptosis. Nat Rev Mol Cell Biol 8: 7

MASCOLO N, SHARMA R, JAIN SC AND CAPAS 1988. Ethnopharmacololgy of *Calotropis proce* ers. J Ethnopharmacol 22: 211–221.

MCCONKEY DJ, LIN Y, NUTT LK, OZEL HZ AN MAN RA. 2000. Cardiac glycosides stimulate C creases and apoptosis in androgen-independent, m human prostate adenocarcinoma cells. Cancer 3807–3812.

MELO MM, VAZ FA, GONÇALVES LC AND SATU HM. 2001. Estudo fitoquímico da *Calotropis proc* sua utilização na alimentação de caprinos: efeitos e bioquímicos séricos. Rev Bras Saúde Prod An 2:

MOREIRA DRM, LEITE ACL, FERREIRA PMP, PM, COSTA-LOTUFO LV, MORAES MO, BR DJ AND PESSOA C. 2007. Synthesis and ant evaluation of peptidyl-like derivatives containing benzodioxole system. Eur J Med Chem 42: 351–

MOSSA JS, TARIQ M, MOHSIN A, AGEEL AM, AL-YAHYA MA, AL-SAID MS AND RAFATULLAH S. 1991. Pharmacological studies on aerial parts of *Calotropis procera*. Am J Chin Med 19: 223–231.

OLIVEIRA JS, BEZERRA DP, FREITAS CDT, FILHO JDBM, MORAES MO, PESSOA C, COSTA-LOTUFO LV AND RAMOS MV. 2007. *In vitro* cytotoxicity against different human cancer cell lines of laticifer proteins of *Calotropis procera* (Ait.) R. Br. Toxicol *in vitro* 21: 1563–1573.

RAMOS MV, BANDEIRA GP, FREITAS CDT, NOGUEIRA NAP, ALENCAR NMN, SOUSA AS AND CARVALHO AFU. 2006. Latex constituents from *Calotropis procera* (R. Br.) display toxicity upon egg hatching and larvae of *Aedes aegypti* (Linn.). Mem Inst Oswaldo Cruz 101: 503–510.

ROY S, SEHGAL R, PADHY BM AND KUMAR VL. 2005. Antioxidant and protective effect of latex of *Calotropis procera* against alloxan-induced diabetes in rats. J Ethnopharmacol 2: 470–473.

SATO DYO, WAL R, DE OLIVEIRA CC, CATTANEO RII, MALVEZZI M, GABARDO J AND BUCHI DF. 2005. Histopathological and immunophenotyping studies on normal and sarcoma 180-bearing mice treated with a complex homeopathic medication. Homeopathy 94: 26–32.

SATYAVATI GV, RAINA MK AND SHARMA M. 1976. Medicinal plants of India. New Delhi, CMR, 19 p.

SCHEUER PJ AND LEFKOWITCH JH. 2000. Drugs and Toxins. In: SCHEUER PJ AND LEFKOWITCH JH (Eds), Liver Biopsy Interpretation, London: WB Saunders, p. 134–150.

SETTY SR, QUERESHI AA, SWAMY AHMV, PATIL T, PRAKASH T, PRABHU K AND GOUDA AV. 2007. Hepatoprotective activity of *Calotropis procera* flowers against paracetamol-induced hepatic injury in rats. Fitoterapia 78: 451–454.

SHARMA P AND SHARMA JD. 2000. *In vitro* schizonticidal screening of *Calotropis procera*. Fitoterapia 71: 77–79.

SMIT HF, WOERDENBAG HJ, SINGH RH, MEULENBELD GJ, LABADIE RP AND ZWAVING JH. 1995. Ayurvedic herbal drugs with possible cytostatic activity. J Ethnopharmacol 47: 75–84.

SUFFNESS M AND PEZZUTO JM. 1990. Assays related to cancer drug discovery. In: HOSTETTMANN K (Ed), Methods in plant biochemistry: assays for bioactivity, London: Academic Press, p. 71–133.

VAN QUAQUEBEKE E, SIMON G, DEWELLE AJ, YAZIDI ME, BRUYNAEEL F, TUTI J, NACOULMA O, GUISSOU P, DECAESTECKER BJC AND KISS RF. 2005. Identification of a novel cardenolide (2″-Oxovoruscharin) from *Calotropis procera* and the hemisynthesis of novel derivatives displaying potent *in vitro* antitumor activities and high *in vivo* tolerance: structure-activity relationship analyses. J Med Chem 48: 849–856.